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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁴ : C12Q 1/68	A1	(11) International Publication Number: WO 87/ 05943 (43) International Publication Date: 8 October 1987 (08.10.87)
(21) International Application Number: PCT/US87/00758 (22) International Filing Date: 26 March 1987 (26.03.87) (31) Priority Application Numbers: 845,610 892,575 (32) Priority Dates: 28 March 1986 (28.03.86) 1 August 1986 (01.08.86) (33) Priority Country: US (71) Applicant: BOARD OF TRUSTEES OF UNIVERSITY OF ILLINOIS [US/US]; Administration Building, 506 South Wright Street, Urbana, IL 61801 (US). (72) Inventors: RONINSON, Igor, B. ; 818 South Laflin Street, Chicago, IL 60607 (US). PASTAN, Ira, H. ; 11710 Beal Mountain Road, Potomac, MD 20854 (US). GOTTESMAN, Michael, M. ; 6400 Maiden Lane, Bethesda, MD 20817 (US).		(74) Agent: GRUBER, Lewis, S.; Marshall, O'Toole, Gerstein, Murray & Bicknell, Two First National Plaza Suite 2100, Chicago, IL 60603 (US). (81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent) Published <i>With international search report.</i>
(54) Title: COMPOSITIONS AND METHODS FOR CLONES CONTAINING DNA SEQUENCES ASSOCIATED WITH MULTIDRUG RESISTANCE IN HUMAN CELLS (57) Abstract Genomic and cDNA clones of human genes which are selectively amplified or overexpressed in multidrug-resistant human tumor cells were isolated. Such clones may be used as probes in diagnostic tests to detect chemotherapy-resistant tumor cells and to predict tumor response to chemotherapy. The complete nucleotide sequence of the coding region of the human <i>mdr1</i> gene and the complete corresponding amino acid sequence are disclosed.		

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COMPOSITIONS AND METHODS FOR CLONES
CONTAINING DNA SEQUENCES ASSOCIATED
WITH MULTIDRUG RESISTANCE IN HUMAN CELLS

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Roninson et al.

This is a Continuation-in-Part of Application
Serial No. 845,610, filed March 28, 1986.

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Background

The present invention pertains in general to
diagnostic materials and methods and in particular to
materials and methods for the detection of multidrug-
15 resistant tumor cells.

Selection of mammalian cells for resistance to
plant alkaloids or antitumor antibiotics frequently
results in the development of cross-resistance to other
drugs unrelated in their structure and mode of action to
20 the original selective agent. Biedler et al., Cancer
Res., 30, 1174 (1970). The phenomenon of multidrug
resistance constitutes a major problem in cancer chemo-
therapy since it involves resistance to some of the most
commonly used anticancer drugs.

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Multidrug resistance in most cases appears to
result from decreased intracellular drug accumulation,
probably as a result of alterations in the plasma mem-
brane. Biedler et al., Cancer Treat. Rep., 67, 859
(1983); Ling et al., Cancer Treat. Rep., 67, 869 (1983);
30 Ramu et al., Cancer Treat. Rep., 67, 895 (1983); and
Beck et al., Cancer Res., 39, 2070 (1979).

In some hamster, mouse and human multidrug-
resistant cell lines, resistance correlates with over
expression of a 170,000 m.w. membrane glycoprotein (P-
35 glycoprotein) or a 19,000 m.w. cytosolic protein. Kart-
ner et al., Science, 221, 1285-1288 (1983); Biedler et

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al., Cancer Treat. Rep., 67, 859 (1983). Immunoblotting techniques applied to cells from human cancer patients reveal high levels of P-glycoprotein in some cases of advanced, nonresponsive ovarian cancer. Bell et al.,
5 J. Clin. Oncol., 3, 311-315 (1985).

P-glycoprotein-specific, monoclonal antibodies raised against multidrug-resistant Chinese hamster ovary (CHO) cell lines and cross reactive with human cell lines apparently bind to multidrug-resistant mammalian
10 cells to a degree correlated with the degree of their drug resistance. Kartner et al., Nature, 316, 820-823 (1985). These monoclonals may all bind to a C-terminal intracellular region of a proposed P-glycoprotein polypeptide. Kartner et al., Nature, 316, 820-823
15 (1985). P-glycoprotein specific cDNA clones have been isolated from Chinese hamster ovary cells, and these clones revealed amplification of the P-glycoprotein gene in multidrug resistant hamster, mouse and human cells when employed in a Southern blotting procedure. Riordan
20 et al., Nature, 316, 817-819 (1985). However, Riordan et al. provides no indication whether the hamster P-glycoprotein cDNA clones may be used to detect the expression of human P-glycoprotein genes at the level of RNA.

25 In a different approach to the examination of multidrug-resistance, a common region of DNA is found to be amplified in two different multidrug-resistant Chinese hamster cell lines selected for resistance to either colchicine or Adriamycin. Roninson et al.,
30 Nature, 309, 626 (1984). This region was found to contain a transcription unit, presently designated mdr. Expression of the mdr mRNA correlates with multidrug resistance in the hamster cells. Gros et al., J. Cell. Biochem., 9C (suppl.), 16, A1167 (1985); and Gros et
35 al., Proc. Natl. Acad. Sci. (USA), 83, 337 (1986). However, probes derived from the hamster mdr gene are

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not useful probes for human cells inasmuch as, even though these probes hybridize to human DNA (as illustrated in Example 2, infra), they do not hybridize efficiently with human mdr mRNA, despite the impression
5 given in a report on a workshop dealing with multidrug resistance [Kolata, Science, 231, 220-221 (1986)].

Therefore, in the absence of a probe for human mdr gene expression, there is a need for a reliable method for detecting the presence of multidrug-resistant
10 cells in a human tumor either prior to or during chemotherapy.

Summary of the Invention

15 The present invention provides an isolated nucleic acid sequence for a human mdr gene associated with multidrug resistance in human cells.

A presently-preferred embodiment of the present invention provides an isolated and purified nucleic
20 acid selected from the group consisting of: (a) a nucleic acid comprising a member of the group consisting of a continuous sequence of nucleotides as set forth in Table 4, in Table 5, in pHDR4.4 (ATCC 40227), in pHDR4.5 (ATCC 40228), in pHDR5A (ATCC 67040), in pHDR5B (ATCC
25 67041), in pHDR10 (ATCC 67042) and in pHDR104 (ATCC 67156); (b) a nucleic acid comprising a nucleotide sequence which hybridizes with at least one of the continuous sequences of nucleotides as set forth in (a) or which is contained within the same mRNA molecule of
30 human origin or cDNA molecule of human origin as at least one of the continuous sequences of nucleotides as set forth in (a); (c) nucleic acids comprising a nucleotide sequence which hybridizes with any nucleotide sequences described in (b); and (d) nucleic
35 acids comprising a sequence of nucleotides which, but for the degeneracy of the genetic code, would hybridize

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with at least one of the continuous sequences of nucleotides as set forth in (a), (b), or (c). Standard conditions for identifying the presence or absence of "hybridization" herein are reactions conducted in 4 X
5 SSC and 0.5% SDS at a temperature of 65 degrees C. in the last wash. A nucleic acid probe according to the preferred embodiment may also include a label associated with one of these nucleic acids. Polypeptides encoded by these nucleic acids may be expressed or synthesized
10 chemically, and used, in conjunction with diluents, adjuvants, or carriers of the sort well known to those skilled in the art, to raise monoclonal or polyclonal antibodies or to elicit immune response in patients. Such antibodies may be utilized as a diagnostic reagent
15 using various presently available immunodiagnostic techniques, or employed as immunotherapeutic agents.

Brief Description of the Drawings

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Fig. 1 is a partial restriction map of the cosmid clone cosDR3A which contains a 5' portion of the transcribed mdr region isolated from Chinese hamster cells;

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Fig. 2 illustrates partial restriction maps of the plasmid clones pHDR4.4 and pHDR4.5, respectively containing mdr1 and mdr2 sequences; and

Fig. 3 illustrates partial restriction maps of phage cDNA clones λ HDR5, λ HDR10, λ HDR62, λ HDR28,
30 λ HDR69A, λ HDR103 λ HDR104 and λ HDR105 containing mdr1 sequences.

Detailed Description

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Preliminary announcements of the obtaining of mdr1 clones according to the present invention and of

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uses therefor have been made by the inventors at the UCLA Symposia on Molecular and Cellular Biology, January 20 - February 15, 1986. Roninson et al., J. Cell. Biochem., 29 (suppl. 10A), 12, A18 (1986); Pastan et al., J. Cell. Biochem., 29 (suppl. 10A), 9, A13 (1986); Clark et al., J. Cell. Biochem., 29 (suppl. 10A), 49, A130 (1986); and Cornwell et al., J. Cell. Biochem., 29 (suppl. 10A), 50, A131 (1986).

A recently published European Patent Application No. 174,180 by John R. Riordan, entitled "Multidrug Resistance in Mammalian Cell Lines And Isolation Of Determinant Glycoprotein DNA," describes isolation of Chinese hamster cDNA clones specific for P-glycoprotein, and it suggests using P-glycoprotein-specific cDNA as a probe in determining multidrug resistance in cells. Although only Southern blot hybridization between hamster cDNA and human genomic DNA is described, claim 18 of Riordan, EPA 174,810, relates to a P-glycoprotein-specific DNA molecule "derived from a source selected from the group consisting of Chinese Hamster Ovary cells, mouse cells and human cells." In the event that the mdr clones described herein represent the human P-glycoprotein gene sequences, which is likely to be the case as discussed in Example 10 below, it should be noted that Riordan, EPA 174,810, does not disclose a human mdr gene or any portion thereof.

In fact, Riordan, EPA 174,810, post-dates the publication of Roninson et al., Nature, 309, 626 (1984) which described cloning of a segment of the Chinese hamster mdr region. The work describe in Roninson et al., Nature, 309, was followed by isolation of the entire Chinese hamster mdr gene [Gros et al., J. Cell. Biochem. and Proc. Nat'l. Acad. Sci. (USA), supra] as opposed to only partial cDNA clones of the Chinese hamster P-glycoprotein genes, as described in Riordan, EPA 174,810. Riordan, EPA 174,810, provides no evidence

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for the ability of Chinese hamster clones to detect the expression of human P-glycoprotein mRNA. Furthermore, the use of P-glycoprotein cDNA as a probe for detection of multidrug resistance in tumor cells is described in Riordan, EPA 174,180, only in terms of detection of amplified P-glycoprotein genes but not in terms of detection of increased P-glycoprotein mRNA expression. Increased mRNA expression, as described in Example 7 below, provides a much more useful diagnostic marker for multidrug resistance than does gene amplification. In addition, although claiming P-glycoprotein cDNA sequences of human origin, Riordan, EPA 174,810, contains no indication as to how such sequences would be obtained, e.g. the source of human DNA or RNA, or stringency conditions for screening of human cDNA or genomic libraries with a Chinese hamster probe. As shown in Example 2 below, there is a low level of homology between the hamster and human mdr genes, at least within the 5' half of the gene, which presents a considerable technical problem in the isolation of human mdr DNA sequences.

In the following examples, nucleic acid clones for human mdr genes and uses for the nucleotide sequences of mdr clones are described. In Example 1 a Chinese hamster mdr clone is used to identify sequences hybridizing with human DNA. Example 2 describes the identification and isolation of DNA sequences comprising human mdr genes. In Example 3, amplification of mdr genes in human drug-resistant cells is demonstrated. A characterization of clones containing mdr sequences is presented in Example 4. In Example 5, DNA rearrangement involving mdr genes is examined. In Example 6, transcription of the mdr1 gene in human cells is demonstrated. Example 7 describes an investigation into expression levels of the mdr1 sequence during the course of development of multidrug resistance in human cells.

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In Example 8, expression of mdr genes out of proportion to gene amplification is demonstrated. Example 9 provides a description of a genomic clone containing a segment of the mdr1 gene. In Example 10, cDNA clones of the mdr1 gene and the cDNA sequence of the human mdr1 gene is disclosed are described. In Example 11, diagnostic and therapeutic procedures using probes according to the present invention are described.

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Example 1

Derivation and characterization of multidrug-resistant sublines of human KB cells are described elsewhere. Akiyama et al., Somat. Cell Mol. Genet., 11, 117 (1985); Fojo et al., Cancer Res., 45, 3002 (1985); and Richert et al., Proc. Natl. Acad. Sci. (USA), 82, 2330 (1985). The multi-drug resistant phenotype is unstable in the most highly resistant lines, with a decrease in resistance when grown in the absence of the drugs. Using the in-gel DNA renaturation technique [according to Roninson, Nucleic Acids Res., 11, 5413 (1983)], several of the multidrug-resistant sublines of KB cells are known to contain amplified DNA sequences, and karyotypic analysis reveal double minute chromosomes in these cells. Fojo et al., Proc. Natl. Acad. Sci. (USA), 82, 7661 (1985).

Sublines of the human KB carcinoma cells, selected for resistance to colchicine, vinblastine or Adriamycin [Akiyama et al., supra; Fojo et al., Cancer Res. supra; Richert et al., supra and Shen et al., Science, 232, 643-645 (1986)], demonstrate the multidrug-resistant phenotype. Several of these sublines are described in Table 1. Fojo et al., Proc. Natl. Acad. Sci. USA, 82, 7661 (1985). In Table 1, "n/d" means not determined. KB-8-5-11, KB-8-5-11-24,

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KB-C3 and KB-C4 cell lines are subclones selected in 100 ng/ml, 1 μ g/ml, 3 μ g/ml and 4 μ g/ml Adriamycin, respectively. Relative resistance is expressed as the D₁₀ of the resistant cell line divided by the D₁₀ of the parental KB-3-1 cells. Akiyama et al., supra.

TABLE 1

		Relative Resistance To:		
10	<u>Cell Line</u>	<u>Colchicine</u>	<u>Adriamycin</u>	<u>Vinblastine</u>
	KB-3-1	1	1	1
	KB-8-5-11	40	23	51
	KB-8-5-11-24	128	26	20
15	KB-C3	487	141	206
	KB-C4	1750	254	159
	KB-C1-R1	6	3	4
	KB-V1	171	422	213
	KB-A1	19	97	43
20	KB-A2	n/d	140	n/d

These multidrug-resistant human KB cell lines were used to determine whether DNA sequences homologous to the hamster mdr gene are present in the human genome. The Chinese hamster mdr DNA sequences used in this study were derived from the cosmid clone cosDR3A, containing a 5' segment of the hamster mdr gene. After digestion with the restriction enzymes XbaI and KpnI, individual 1.5 - 6 kilobase (kb) restriction fragments from this cosmid were either subcloned into pSP65 plasmid vector commercially available from Promega Biotec, Madison, Wisconsin, or gel-purified prior to labeling with ³²P. A vector including a 4.7 kb XbaI fragment, designated pDR4.7, contained DNA sequences hybridizing to human DNA.

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In Fig. 1, a partial restriction endonuclease map of the cosmid clone cosDR3A, containing a 5' portion of the transcribed mdr region amplified in multidrug-resistant Chinese hamster cells, is presented along with a dashed line aligned to indicate the portion of ROS DR3A which hybridizes to pDR4.7. In Fig. 1, X denotes an XbaI site and K identifies a KpnI site. Cloning and characterization of this region are described in Gros et al., Proc. Natl. Acad. Sci. USA, 83, 337 (1986).

10

Example 2

In order to identify and isolate segments of DNA comprising the human mdr genes, individual 1.5 - 6 kilobase (kb) size fragments of the cloned hamster mdr gene were isolated as a series of recombinant subclones in a pSP64 plasmid vector commercially available from Promega Biotec and described in Promega Biotec Technical Bulletin No. 13 as well as in Melton, Nucleic Acids Res., 12, 7055-7056 (1984). Individual subclones were then labeled with ³²P and were used as probes for Southern blot hybridization with human DNA digested with restriction enzymes.

The subclones were then used as probes for hybridization with restriction digests of human genomic DNA. Most probes, when used under conditions of low hybridization stringency, produced either no hybridization signal or a continuous smear suggesting cross-hybridization with human repetitive DNA sequences.

However, one of the subclones, designated pDR4.7 and illustrated in Fig. 1, gave rise to distinct bands when hybridized to human DNA under low stringency conditions.

Inasmuch as subclone pDR4.7, produced a distinct hybridization signal, this subclone contained hamster DNA sequences homologous to the human mdr genes. pDR4.7 hybridized to two major different EcoRI

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SSC; 65°C). Only the 13.5 kb EcoRI and 4.4 kb HindIII fragments were detectable under conditions of intermediate stringency (1 x SSC; 65°C). All the fragments were amplified in colchicine-resistant sublines

5 KB-8-5-11, KB-8-5-11-24, KB-C3 and KB-C4.

No amplification of either the band corresponding to the 13.5 kb fragment or the band corresponding to the 4.4 kb fragment was detected in the revertant subline KB-C1-R1. Unlike the colchicine-selected sub-
10 lines, the subline KB-V1, selected in vinblastine, shows amplification of only the 13.5 kb EcoRI and the 4.4 kb HindIII bands. These two bands were also amplified in Adriamycin-resistant cells KB-A1 and KB-A2. KB-A1, in addition, contained a new amplified band of a 7 kb size
15 in the EcoRI digest and of a 6.5 kb size in the HindIII digest. The same bands were present in KB-V1 DNA, but their intensity suggested that these bands were not amplified. No bands of this size were detected in the parental KB-3-1 DNA, suggesting that they apparently
20 arose as a result of a DNA rearrangement.

The different patterns of amplification of the two types of bands hybridizing to the hamster mdr probe in different sublines suggested that they might correspond to two different related DNA sequences, possibly
25 different members of a multigene family, rather than to two different parts of one contiguous hybridizing region. DNA sequences corresponding to the 13.5 kb EcoRI and 4.4 kb HindIII fragments were designated mdr1 and the sequences corresponding to the 4.5 kb EcoRI and
30 the 10.5 kb HindIII fragments were designated mdr2.

The degree of amplification of mdr sequences in different multidrug-resistant sublines was estimated by comparing the intensity of hybridization signals from serially diluted EcoRI digests of different cellular
35 DNAs. The estimates of the copy number of mdr sequences in different sublines are given in Table 2. In Table 2,

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a star indicates the rearrangement of mdr2 DNA sequences.

TABLE 2

5	<u>Cell Line</u>	Degree of Amplification	
		<u>mdr1</u>	<u>mdr2</u>
	KB-3-1	1	1
10	KB-8-5-11	7-8	7-8
	KB-8-5-11-24	9	9
	KB-C3	20	20
	KB-C4	30	30
	KB-C1-R1	1	1
15	KB-V1	100	1*
	KB-A1	70	30*
	KB-A2	80	1

By comparison of Table 1 with Table 2, it may be observed that in the sublines selected for a 40-700 fold degree of resistance to colchicine, there is a general, but not precise, correlation between increases in drug resistance and in the copy number of mdr sequences. The degree of resistance may correlate more precisely with the expression of mdr RNA than with the degree of mdr gene amplification. The mdr1 and mdr2 sequences appear to be amplified to a similar degree in these cells. The loss of amplified mdr sequences in a revertant of a colchicine-resistant cell line provides strong additional evidence that mdr gene amplification underlies multidrug resistance in the highly resistant cells. The degree of amplification of mdr1 in the cells selected for resistance to vinblastine or Adriamycin appears to be higher than in the cells with a similar degree of resistance that have been selected with colchicine.

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Example 4

To investigate the nature of the human mdr genes, clones containing mdr1 and mdr2 sequences were isolated from the DNA of the colchicine-resistant sub-line KB-C3. For this purpose, two phage libraries containing complete EcoRI or HindIII digests of KB-C3 DNA were prepared. The EcoRI library was constructed by insertion into the EcoRI site of the λ gt11 phage vector, and the HindIII library was made by insertion into the HindIII site of Charon 28 [Young et al., Proc. Natl. Acad. Sci. (USA), 80, 1194 (1983); Rimm et al., Gene, 12, 301 (1980)]. Both libraries were screened by plaque hybridization with the Chinese hamster pDR4.7 probe according to the procedure of Benton et al., Science, 196, 180 (1977). A clone containing the 4.4 kb HindIII fragment (mdr1) was isolated from the HindIII library, and a clone containing the 4.5 kb EcoRI fragment (mdr2) was isolated from the EcoRI library. Both inserts were subsequently recloned into the plasmid vector pSP64 [Melton et al., Nucleic Acids Res., 12, 7035 (1984)], giving rise to plasmid clones designated pHDR4.4 and pHDR4.5, respectively. Plasmid clone pHDR4.4 was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, as Deposit No. 40227 on March 21, 1986. Likewise, plasmid clone pHDR4.5 was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, as Deposit No. 40228 on March 21, 1986. Partial restriction maps of these clones are shown in Fig. 2. In Fig. 2, sites for digestion by corresponding restriction endonucleases are identified as follows: "A", AvaI; "B", BamHI; "E", EcoRI; "G", BglII; "H", HindIII; "J", HaeII; "P", PstI; "V", PvuII; and "X", XbaI.

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In Fig. 2, solid bars indicate the fragments containing highly repeated sequences. These fragments were identified by hybridization of Southern blots containing restriction digests of cloned DNA with 0.35×10^5 dpm/cm² of ³²P-labeled total human genomic DNA. Dashed lines indicate the DNA sequences hybridizing to the pDR4.7 clone, as determined by Southern hybridization with the gel-purified pDR4.7 insert.

Because the pDR4.7 hamster probe was known to contain transcriptionally active sequences expressed in multidrug-resistant hamster cells [Gros et al., Proc. Nat'l. Acad. Sci. (USA), supra] it seemed likely that the conserved human mdr sequences would provide convenient probes for transcription studies. The hamster pDR4.7 probe hybridized very poorly, if at all, to mRNA from multidrug-resistant human cells, and therefore could not be used as a probe for detection of mdr genes in human cells. Consequently, repeat-free fragments of both clones which hybridized to pDR4.7 were subcloned into the plasmid vector pSP64. The clone containing a 0.75 kb PvuII fragment of pHDR4.4, inserted into the SmaI site of the vector, was designated pMDR1. The clone containing a 1.0 kb PstI fragment of pHDR4.5, inserted into the PstI site of the vector, was designated pMDR2. These two clones were found to cross-hybridize with each other under conditions of low hybridization stringency providing additional evidence that mdr1 and mdr2 represent related DNA sequences.

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Example 5

To determine whether the rearranged bands in KB-V1 and KB-A1 correspond to mdr1 or mdr2, DNA from different sublines was digested with HindIII and hybridized to either hamster pDR4.7 probe or to the human

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pMDR1 or pMDR2 probes. Hybridization with the gel-purified insert of the plasmid pDR4.7 was done under conditions of low stringency (4 X SSC, 0.5% SDS at 65°C). The same blot was then rehybridized with gel-purified
5 inserts of the plasmids pMDR1 and pMDR2 under high stringency conditions (0.1 X SSC, 0.5% SDS at 65°C) so that the signal resulting from cross-hybridization of mdr1 and mdr2 sequences was minimized.

This experiment demonstrated that rearranged
10 bands in both KB-A1 and KB-V1 sublines correspond to mdr2. The mobility of the new bands appears to be identical in several different restriction digests of KB-V1 and KB-A1 DNA, indicating that a similar rearrangement may have occurred in both independently selected sub-
15 lines. However, while the rearranged bands are amplified in KB-A1, they do not appear amplified in KB-V1 cells. In addition, both types of cells contain bands corresponding to the unrearranged allele of mdr2, which is not amplified. Amplification of the rearranged but
20 not the parental mdr2 band in KB-A1 cells suggests that DNA rearrangement either preceded or occurred simultaneously with the onset of gene amplification in these cells. In the case of KB-V1, it is unclear whether mdr2 rearrangement is related to amplification of mdr1.

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Example 6

To determine whether the evolutionarily conserved regions of mdr1 and mdr2 contained transcribed
30 sequences, pMDR1 and pMDR2 were used as probes for Northern hybridization, performed according to the procedure of Thomas, Proc. Natl. Acad. Sci. (USA), 77, 5201-5205 (1980) with poly (A)⁺ RNA extracted from the parental KB-3-1 and multidrug-resistant KB-C2.5 cells
35 [Akiyama et al., supra; Fojo et al., Cancer Res., supra; Richert et al., supra and Shen et al., supra] under the

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conditions of high hybridization stringency as recited in Example 5. Poly (A)⁺ RNA was extracted from the parental drug-sensitive KB-3-1 cells and from the colchicine-resistant KB-C2.5 subline as described in Chirgwin et al., Biochem., 18, 5294 (1979). One microgram of each RNA preparation was electrophoresed in a 1.5% glyoxal agarose gel [McMaster et al., Proc. Natl. Acad. Sci. (USA), 74, 4835 (1977)] and transferred onto Gene Screen Plus™ membrane as available from New England Nuclear, Boston, MA. The membranes were hybridized with 3 x 10⁵ dpm/cm² of pMDR1 or pMDR2 probes. Hybridization was done in 1M NaCl, 10% dextran sulphate, 1% SDS, 50% formamide, 100 µg/ml denatured salmon sperm DNA at 42°C. The membranes were washed with 0.1 x SSC, 0.5% SDS at 65°C and autoradiographed. The size of the RNA band was determined relative to the positions of 28S and 18S ribosomal RNA.

The probe pMDR1 hybridizes to an mRNA band of a 4.5 kb size which is highly expressed in the drug-resistant cells. This mRNA is not detectable in the parental KB-3-1 cells, indicating little or no expression when the probes were labelled either by nick translation or oligolabelling. No distinct bands, however, could be detected when pMDR2 was used as a probe. In addition, no bands were revealed by using other repeat-free subfragments of pHDR4.5 as probes in addition to pMDR2. While the existence of transcriptionally active sequences in other regions of mdr-2 or transcription of mdr2 sequences at a very low level cannot be excluded by these results, transcription of the amplified region of mdr2 homologous to the Chinese hamster mdr gene is not detected by Northern hybridization.

Amplification and over expression of DNA sequences homologous to the Chinese hamster mdr gene in multidrug-resistant human KB carcinoma cells suggests that a similar mechanism may be responsible for multidrug resistance in both human and rodent cells. The

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nature of the proteins encoded by mdr genes is still unknown. The size of mdr1 mRNA is consistent with the possibility that it may code for a 170 kd glycoprotein overexpressed in various multidrug-resistant cell lines
5 [Biedler et al., supra; Ling et al., supra; Ramu et al., supra; Beck et al., supra; Kartner et al., Science, 221, 1285 (1983); Debenham et al., Mol. Cell. Biol., 2, 881 (1982); Robertson et al., Mol. Cell. Biol., 4, 500 (1984)]. It is also unknown whether the same mechanism
10 is utilized in the development of multidrug resistance by human tumor cells in vitro and in the course of chemotherapy. The availability of cloned probes which detect transcription of mdr DNA in human cells makes it possible now to investigate expression of these
15 sequences in clinical samples of multidrug-resistant tumors.

Example 7

20 In order to examine levels of expression of mdr1 sequences during the development of multidrug resistance, multidrug-resistant sublines of human KB carcinoma cells and two other human multidrug resistant cell lines of different origin were studied.

25 Agents used in selecting different sublines in multiple steps were colchicine, Adriamycin and vinblastine. In the first two steps of colchicine selection, clones were only obtained if the cell populations were first mutagenized with ethylmethane sulfonate (EMS).
30 Similarly, KB cell lines selected independently for resistance to Adriamycin or vinblastine [Akiyama et al., supra; Fojo et al., Cancer Res., supra; Richert et al., supra; and Shen et al., supra] were obtained only after mutagenesis with EMS in the first step. Subsequent
35 selection, up to very high levels of resistance, was possible without mutagenesis, and occurred at high

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frequency.

The isolation and some properties of the human multidrug resistant KB carcinoma cell lines has been previously described in Akiyama et al., supra; Fojo et al., Cancer Res., supra; and Richert et al., supra. The KB cell lines used in this study, the manner of their selection, and their relative resistance to various drugs, are shown in Table 3. CEM is a cell line described in Beck in Advances in Enzyme Regulation, 22, G. Weber, ed. (Pergamon Press, Oxford, 1984), 207, and 2780 is a cell line described in Rogan et al., Science, 224, 994 (1984).

To determine the extent to which mdr1 sequences were expressed in these cell lines and the size of the corresponding RNAs, a Northern hybridization was performed with total RNA and poly (A)⁺-RNA from these cells. A 4.5 kilobase RNA, which migrates just below the 28S ribosomal RNA marker, was clearly visible in all the lanes containing either total or poly (A)⁺ RNA from the resistant lines but was not seen in any of the sensitive cell lines.

Slot blot hybridization of total RNA was used to quantitate the expression of mdr1 in various sensitive and resistant cell lines. RNA prepared as previously described above was applied to filters using a Schleicher and Schuell slot blot apparatus or by blotting after electrophoresis in 1% agarose containing 13.4% formaldehyde. A gel-purified insert from the pMDR1 clone was ³²P-labeled for use as a probe. Nitrocellulose filters were baked and preincubated for 4-6 hours at 42°C in 50% formamide, 5 x SSC, 10X Denhardt's solution, 0.1% SDS and 100 µg/ml salmon sperm DNA. Filters were hybridized overnight in the above solution containing ³²P-labeled probe. Filters were washed 3 times for 10 minutes at room temperature in 2 x SSC, 0.1% SDS and 3 times for 20 minutes at 50°C in 0.1 x

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SSC, 0.1% SDS. Levels of mdr1 expression were determined by densitometry of the autoradiograms. Tracings of peaks were cut out and weighed and compared to the KB-8 peak which was arbitrarily assigned a value of 1.

5 The results are presented in Table 3 along with the relative drug resistances of the human leukemic lymphoblast cell lines, and the human ovarian cancer cell lines used in the study. In Table 3, ND is an abbreviation for "none detected".

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TABLE 3

Cell Line	Selecting Agent	Relative Resistance to			<u>mdrl mRNA Expression</u>
		<u>Col</u>	<u>Adr</u>	<u>Vbl</u>	
KB-3-1	parental KB	1	1	1	ND
KB-8	colchicine, 5 ng/ml	2.1	1.1	1.2	1
KB-8-5	colchicine, 10 ng/ml	3.8	3.2	6.3	3
KB-8-5-11	colchicine, 100 ng/ml	40	23	51	80
KB-C1	colchicine, 1 µg/ml	260	160	96	270
KB-C1-R1	revertant of KB-C1	6	3	4	1
KB-C1.5	colchicine, 1.5 µg/ml	320	--	140	340
KB-C6	colchicine, 6 µg/ml	2,100	320	370	820
KB-A1	Adriamycin, 1 µg/ml	19	97	43	270
KB-V1	vinblastine, 1 µg/ml	170	420	210	320
CEM	parental leukemic	1	1	1	ND
CEM-V1b ₁₀₀	vinblastine	45	120	420	250
2780	parental, ovarian	1	1	1	ND
2780-Ad	Adriamycin	--	170	15	260

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As shown in Table 3, there was a good correlation between extent of multidrug resistance and the level of mdr1-specific mRNA. As can also be seen in Table 3, there is little or no expression of the mdr1 sequences in parental, drug-sensitive cell lines, but increasing expression occurs as the cell lines become more resistant to drugs. A revertant cell line, KB-C1-R1, subcloned in the absence of colchicine from the colchicine-resistant cell line KB-C1, still expresses mdr1 sequences at reduced levels consistent with its low level of multidrug resistance.

It is not possible to calculate the exact extent of increased expression in the resistant cell lines relative to the parental line, since the hybridization signal from the parental RNA was too weak. However, the extent of expression relative to the KB-8 cell line has been calculated and these data are shown in Table 3. Expression appears to correlate well with increasing drug-resistance for every step of selection in KB cells and reaches very high levels in our most resistant KB cell lines.

The data summarized in Table 3 indicate that two other human cell lines of different origin, selected for multidrug resistance, also express high levels of the 4.5 kb mRNA. Very little or no expression of this RNA was detected in the parental cell lines. The human leukemic lymphoblast cell line CEM (A.T.C.C. CCL119) and its resistant derivatives CEM-VLB₁₀₀, selected for resistance to vinblastine (gift of W. Beck, St. Jude's Hospital) (Beck, supra.) and the ovarian cell line 2780 and its resistant derivative 2780-Ad, selected for resistance to Adriamycin (gift of T. Hamilton and R. Ozols, National Institutes of Health) (Rogan et al., supra) both showed high levels of expression of the 4.5 kb mRNA. Because even low levels of cellular multidrug-resistance may result in clinically refractory tumors,

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expression of mdr1 mRNA in sublines having a low level (2-6 fold) of relative drug resistance but not in the parental drug-sensitive cell lines is of particular interest. In this regard the results presented in Table 5 3 indicate that quantitation of mdr1 mRNA expression may potentially be used for diagnosis of multidrug resistance in clinical tumor specimens.

Example 8

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To compare the levels of mdr1 mRNA expression with the extent of amplification of the genomic mdr1 sequences genomic DNA was isolated from all of the cell lines described in Example 6. Following digestion with 15 HindIII, amplification of mdr1 was examined by Southern blot analysis.

DNA, prepared as previously described in Example 3, was digested with HindIII and electrophoresed in 0.8% agarose gels before Southern transfer to Gene 20 Screen Plus™ (New England Nuclear). The blots were hybridized with the pMDR1 probe for 18 hours at 42°C in 50% formamide, 5 x SSC, 1% SDS with 100 µg/ml salmon sperm DNA. The blots were then washed with 2 x SSC at room temperature for 10 minutes, 2 x SSC, 1% SDS at 42°C 25 for 60 minutes and 0.1 x SSC at room temperature for 60 minutes prior to autoradiography.

No amplification of mdr1 was found in the KB cell lines with low levels of resistance (KB-8, KB-8-5 and the revertant subline, KB-C1-R1), even though these 30 cell lines expressed increased levels of mdr1 mRNA. Increased expression of mdr1 sequences in human cells may therefore occur prior to gene amplification. Amplification of the mdr1 gene was detected in highly resistant sublines of KB cells selected in colchicine, vinblastine or Adriamycin, as well as in CEM-VLB₁₀₀ and 35 2780-Ad cell lines. In the latter two sublines, the

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degree of gene amplification was estimated by densitometry to be approximately 5-10 fold for 2780-Ad and 10-15 fold for CEM-VLB₁₀₀.

In all cases, the increase in mRNA expression was clearly greater than the extent of amplification. These results suggest that the evolution of these lines involved a step or steps in which expression was increased out of proportion to gene amplification. A similar dissociation of amplification and expression of the dhfr gene has been reported for human cancer cells selected for resistance to methotrexate in vitro. [Frei et al., Proc. Natl. Acad. Sci. (USA), 81, 2873 (1984); Wolman et al., Proc. Natl. Acad. Sci. (USA), 80, 807 (1983).] The development of multidrug resistance in human KB cells differs in this respect from Chinese hamster V79 cells where a low (5-7 fold) degree of relative drug resistance is accompanied by 5-10 fold amplification of mdr DNA [Roninson et al., supra; and Gros et al., supra].

These studies demonstrate a correlation between expression of the mdr1 gene and the development of resistance to multiple agents in five independently-derived human cell lines of different origins selected for resistance to different cytotoxic drugs. Expression of mdr1 may therefore represent a common mechanism of multidrug resistance in human cell lines. Increased expression of mdr1 in at least some cases occurs initially without gene amplification and may be a prerequisite for the development of multidrug resistance. This observation may be especially relevant for the analysis of the role of the mdr1 gene in the development of multidrug resistance by human tumors in the course of chemotherapy and may have diagnostic potential. Since the tumor cells are expected to have a relatively low degree of resistance, such an analysis may involve quantitation of mdr1 RNA expression rather than gene amplification in tumor samples.

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Example 9

The segment of the mdr1 gene cloned into pMDR1
5 was sequenced by the chemical degradation procedure
[Maxam et al., Meth. Enzymol., 65, 499, (1980)] and the
enzymatic chain-termination sequencing technique [Sanger
et al., Proc. Natl. Acad. Sci. USA, 74, 5463, (1977)]
using supercoiled plasmid DNA as a template [Zagursky et
10 al., Gene Anal. Techn., 2, 89, (1985)]. To facilitate
sequencing, pMDR1 was mapped with HaeIII and RsaI and
individual 220-400 bp fragments of pMDR1 were subcloned
into a pUC18 plasmid vector (Bethesda Research
Laboratories, Rockville, MD). The sequence of pMDR1 was
15 confirmed by sequencing both strands. The complete
sequence of pMDR1 is presented in Table 4. Comparison
with the sequence of the corresponding cDNA clones in
Example 10 below indicated that pMDR1 includes segments
of two protein-coding sequences (exons), comprising
20 nucleotides 1-111 and 653-807, and an intervening
sequence (intron) which is not expressed as mRNA and
which comprises nucleotides 112-652. Table 4 shows that
amino acid sequence corresponding to the exons within
pMDR1. This amino acid sequence therefore defines a
25 segment of the mdr1 protein product.

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TABLE 4

SEQUENCE OF THE pMDR1 CLONE

1	T	GGA	AGA	CAA	ATA	CAC	AAA	ATT	AGA	AAA	CAG	TTT	TTT	CAT	GCT
		Gly	Arg	Gln	Ile	His	Lys	Ile	Arg	Lys	Gln	Phe	Phe	His	Ala
44		ATA	ATG	CGA	CAG	GAG	ATA	GGC	TGG	TTT	GAT	GTG	CAC	GAT	GTT
		Ile	MET	Arg	Gln	Glu	Ile	Gly	Trp	Phe	Asp	Val	His	Asp	Val
86		GGG	GAG	CTT	AAC	ACC	CGA	CTT	ACA	GA					
		Gly	Glu	Leu	Asn	Thr	Arg	Leu	Thr	As					
112															
151		TATCCCTTAGT	AAAATGAAAT	AGATCTCATC	ACATCTGTTA	GGAGTGTTA									
201		ATGTATCATT	CAAAGGTACT	TATGAGACAA	AATTCCTTCT	AAGCAGCAAC									
251		AATGTCCGTGT	GCATCCCTTT	GTCCCCAGTG	CCTTGACAGG	GTATGGGGGG									
301		ACCTGCATGA	CTAGCATTA	ATGAAGGACT	GGCTTTCCA	GAATGAAGAA									
351		ATCCTCTGAG	AATGTGCAGT	AGAGCAAAAC	AAGATACTTT	CTGAGGAAAT									
401		TTCCTGAGCAA	TTTGAAATTC	CTAGGTTGAA	TACTTCTTGT	GTACACGATG									
451		TCCATTTCCT	GGGGCCATGT	GGCTATGCAT	TCTTGTGTT	AATGACAAAT									
501		ATCCTAGTAG	AAACTTCTAC	CCTGCTAAAT	AAAACAAAGC	ATAGGCACAA									
551		AATACTCTAG	CCATAAACTA	CCCTACACTC	AAAACAGGCT	TCACGAGAAA									
601		AGTTGATGTT	TACAATTCTG	ACAATTATTT	CTAACACTAT	CTGTTCTTTC									
651		AG													
653	T	GAT	GTC	TCT	AAG	ATT	AAT	GAA	GTT	ATT	GGT	GAC	AAA	ATT	GGA
		p	Asp	Val	Ser	Lys	Ile	Asn	Glu	Val	Ile	Gly	Asp	Lys	Ile
696		ATG	TTC	TTT	CAG	TCA	ATG	GCA	ACA	TTT	TTC	ACT	GGG	TTT	ATA
		MET	Phe	Phe	Gln	Ser	MET	Ala	Thr	Phe	Phe	Thr	Gly	Phe	Ile

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TABLE 4 (cont'd.)

738	GTA GGA TTT ACA CGT GGT TCG AAG CTA ACC CTT GTG ATT TTG Val Gly Phe Thr Arg Gly Trp Lys Leu Thr Leu Val Ile Leu	
780	GCC ATC AGT CCT GTT CTT GGA CTG TCA G Ala Ile Ser Pro Val Leu Gly Leu Ser	807

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Example 10

In order to isolate cDNA clones of the mdr1 gene, poly(A)+ RNA was isolated as described in Chirgwin et al., Biochemistry, 18, 5294 (1979) and Aviv et al., Proc. Natl. Acad. Sci. (USA), 69, 1408 (1972) from the subline KB-C2.5, selected with colchicine. A cDNA library was constructed using the steps of synthesizing double-stranded cDNA, blunt ending, attachment of EcoRI linkers and insertion into the phage vector λ gt11 [Young and Davis, supra; Huynh et al., in: DNA Cloning Techniques: A Practical Approach, D. Glover, ed., IRL Press, Oxford, (1985)]. The cDNA library was screened by plaque hybridization (Benton et al., supra) with the pMDR1 probe. Approximately 120 positive clones were isolated. The inserts from five of these clones (λ HDR5, λ HDR10, λ HDR28, λ HDR62 and λ HDR69) were re-cloned into plasmid vectors pGEM1 and pGEM4 (Promega Biotec). The partial restriction maps of these clones are shown in Fig. 3. DNA from λ HDR5 was treated with EcoRI which generated two fragments, designated 5A and 5B. The fragments were subcloned into pGEM1 at its EcoRI site to give plasmids pHDR5A and pHDR5B which were deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, on March 18, 1986, and which received the respective accession numbers ATCC 67040 and ATCC 67041. Similarly, λ HDR10 was treated with EcoRI and cloned into the EcoRI site of pGEM1 to produce pHDR10 which was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, on March 18, 1986, as Deposit No. 67042.

To isolate the remaining portion of mdr1 cDNA, a fragment of the clone λ HDR5, indicated with a striped bar in Fig. 3, was used to screen the same cDNA library. The inserts from three of the positive clones, designated λ HDR10, λ HDR10, and λ HDR10,

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into the EcoRI sites of plasmid vectors pGEM1 and pGEM4, giving rise to plasmids designated pHDR103, pHDR104 and pHDR105, respectively. The plasmid pHDR104 was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, on July 16, 1986, as Deposit No. 67156.

A comparison of the restriction maps of individual clones indicates divergence in the cDNA structure among, for example, clones λ HDR10, λ HDR28 and λ HDR69. The most highly conserved region among these clones is represented by a 270 bp PvuII fragment, which corresponds to the exon regions of pMDR1 and is indicated with a solid bar above the lines in Fig. 3. The variant sequences specific to clones λ HDR62 and λ HDR105 were detected by DNA sequencing, and they are shown as solid bars underneath the corresponding lines in Fig. 3. In Fig. 3, sites for digestion by corresponding restriction endonucleases are identified as follows: "A", AccI; "E", EcoRI; "H", HindIII; "N", XmnI; "P", PvuII; "S", StuI; "T", SstI; "V", AvaI; and "X", XbaI.

The cDNA clones λ HDR10, λ HDR5 and λ HDR104 were sequenced in their entirety using the methods of subcloning the inserts into an M13 phage vector [Messing, Meth. Enzymol., 101, 20, 1983], generating a series of overlapping deletion subclones [Henikoff, Gene, 28, 351, 1984] and determining their DNA sequence by the enzymatic chain-termination sequencing techniques [Sanger et al., supra]. A part of the cDNA sequence was determined by specific-primer-directed DNA sequencing [Strauss et al., Anal. Biochem., 154, 353 1986] using supercoiled plasmid DNA as a template [Zagursky et al., supra]. The overlapping regions of clones λ HDR10, λ HDR5 and λ HDR104 were found to be identical, and therefore, these clones are assumed to represent different parts of the same cDNA. The combined cDNA sequence of clones λ HDR10, λ HDR5 and λ HDR104 is shown in Table 5. This

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table also shows the amino acid sequence of mdr1 gene product, derived from the same cDNA sequence.

TABLE 5

MDR1 cDNA SEQUENCE (CLONES λHDR10; λHDR5; λHDR104)

1	CCTACTCTAT	TCAGATATTC	TCCAGATATCC	TAAAGATTAG	AGATCATTTTC	
51	TCATTCCTCT	AGGAGTACTC	ACTTCAGGAA	GCAACCAGAT	AAAAGAGAGG	
101	TGCAACGGAA	GCCAGAACAT	TCCTCCTGGA	AATTCAACCT	GTTTCGCAGT	
151	TTCTCGAGGA	ATCAGCATTC	AGTCAATCCG	GGCCGGGCGT	AGTCATCTGT	
201	GGTGAGGCTG	ATTGGCTGGG	CAGGAACAGC	GCCGGGGCGT	GGGCTGAGCA	
251	CAGCGCTTCG	CTCTCTTTGC	CACAGCAAGC	CTGAGCTCAT	TCGAGTAGCG	
301	GCTCTTCCAA	GCTCAAAGAA	GCAGAGGCCG	CTGTTTCGTTT	CCTTTAGGTC	
351	TTTCCACTAA	AGTCGGAGTA	TCTTCTTCCA	AGATTTCACG	TCTTGGTGGC	
401	CGTTCCAAGG	AGCGCGAGGT	CGGG			
425	ATG GAT CTT	GAA GGG GAC CGC	AAT GGA GGA GCA	AAG AAG AAG AAG		466
	MET Asp	Leu Glu Gly Asp	Arg Asn Gly Ala	Lys Lys Lys Lys		
467	AAC TTT TTT	AAA CTG AAC AAT	AAA AGT GAA AAA	GAT AAG AAG AAG		508
	Asn Phe	Lys Leu Asn Asn	Lys Ser Glu Lys	Asp Lys Lys Lys		
509	GAA AAG AAA	CCA ACT GTC AGT	GTA TTT TCA	ATG TTT CGC TAT		550
	Glu Lys Lys	Pro Thr Ser Val	Phe Ser MET	Phe Arg Tyr		
551	TCA AAT TGG	CTT GAC AAG TTG	TAT ATG GTG	GGA ACT TTG		592
	Ser Asn	Trp Leu Asp Lys	Leu Tyr MET	Val Val Gly Thr	Leu	
593	GCT GCC ATC	ATC CAT GGG GCT	GGA CTT CCT	CTC ATG ATG	CTG	634
	Ala Ala	Ile Ile His Gly	Ala Gly Leu	Pro Leu MET	Leu	
635	GTG TTT GGA	GAA ATG ACA	GAT ATC TTT	GCA AAT GCA	GGA AAT	676
	Val Phe	Gly Glu MET	Thr Asp Ile	Phe Ala Asn	Gly Asn	

TABLE 5 (cont'd.)

677	TTA GAA GAT CTG ATG TCA AAC ATC ACT AAT AGA AGT GAT ATC Leu Glu Asp Leu MET Ser Asn Ile Thr Asn Arg Ser Asp Ile	718
719	AAT GAT ACA GGG TTC TTC ATG AAT CTG GAG GAA GAC ATG ACC Asn Asp Thr Gly Phe MET Asn Leu Glu Asp MET Thr	760
761	AGG TAT GCC TAT TAT TAC AGT GGA ATT GGT GCT GGG GTG CTG Arg Tyr Ala Tyr Tyr Tyr Ser Gly Ile Gly Ala Gly Val Leu	802
803	GTT GCT TAC ATT CAG GTT TCA TTT TGG TGC CTG GCA GCT Val Ala Ala Tyr Ile Gln Val Ser Phe Trp Cys Leu Ala Ala	844
845	GCA AGA CAA ATA CAC AAA ATT AGA AAA CAG TTT TTT CAT GCT Gly Arg Gln Ile His Lys Ile Arg Lys Gln Phe Phe His Ala	886
887	ATA ATG CGA CAG GAG ATA GGC TGG TTT GAT GTG CAC GAT GTT Ile MET Arg Gln Gln Ile Gly Trp Phe Asp Val His Asp Val	928
929	GGG CAG CTT AAC ACC CGA CTT ACA GAT GAT GTC TCT AAG ATT Gly Glu Leu Asn Thr Arg Leu Thr Asp Asp Val Ser Lys Ile	970
971	AAT CAA GTT ATT GGT GAC AAA ATT CGA ATG TTC TTT CAG TCA Asn Glu Val Ile Gly Asp Lys Ile Gly MET Phe Phe Gln Ser	1012
1013	ATG GCA ACA TTT TTC ACT GGG TTT ATA GTA GGA TTT ACA CGT MET Ala Thr Phe Phe Thr Gly phe Ile Val Gly phe Thr Arg	1054
1055	GGT TGG AAG CTA ACC CTT GTG ATT TTG GCC ATC AGT CCT GTT Gly Trp Lys Leu Thr Leu Val Ile Leu Ala Ile Ser Pro Val	1096

TABLE 5 (cont'd.)

1097	CTT	GGA	CTG	TCA	GCT	GCT	GTC	TGG	GCA	AAG	ATA	CTA	TCT	TCA	1138
	Leu	Gly	Leu	Ser	Ala	Ala	Val	Trp	Ala	Lys	Ile	Leu	Ser	Ser	
1139	TTT	ACT	GAT	AAA	GAA	CTC	TTA	GCG	TAT	GCA	AAA	GCT	GGA	GCA	1180
	Phe	Thr	Asp	Lys	Glu	Leu	Leu	Ala	Tyr	Ala	Lys	Ala	Gly	Ala	
1181	GTA	GCT	GAA	GAG	GTC	TTG	GCA	GCA	ATT	AGA	ACT	GTG	ATT	GCA	1222
	Val	Ala	Glu	Glu	Val	Leu	Ala	Ala	Ile	Arg	Thr	Val	Ile	Ala	
1223	TTT	GGA	GGA	CAA	AAG	AAA	GAA	CTT	GAA	AGG	TAC	AAC	AAA	AAT	1264
	Phe	Gly	Gly	Gln	Lys	Lys	Glu	Leu	Glu	Arg	Tyr	Asn	Lys	Asn	
1265	TTA	GAA	GAA	GCT	AAA	AGA	ATT	GGG	ATA	AAG	AAA	GCT	ATT	ACA	1306
	Leu	Glu	Glu	Ala	Lys	Arg	Ile	Gly	Ile	Lys	Lys	Ala	Ile	Thr	
1307	GCC	AAT	ATT	TCT	ATA	GGT	GCT	GCT	TTC	CTG	CTG	ATC	TAT	GCA	1348
	Ala	Asn	Ile	Ser	Ile	Gly	Ala	Ala	Phe	Leu	Leu	Ile	Tyr	Ala	
1349	TCT	TAT	GCT	CTG	GCC	TTC	TGG	TAT	GGG	ACC	ACC	TTG	GTC	CTC	1390
	Ser	Tyr	Ala	Leu	Ala	Phe	Trp	Tyr	Gly	Thr	Thr	Leu	Val	Leu	
1391	TCA	GGG	GAA	TAT	TCT	ATT	GGA	CAA	GTA	CTC	ACT	GTA	TTC	TTT	1432
	Ser	Gly	Glu	Tyr	Ser	Ile	Gly	Gln	Val	Leu	Thr	Val	Phe	Phe	
1433	TCT	GTA	TTA	ATT	GGG	GCT	TTT	AGT	GTT	GGA	CAG	GCA	TCT	CCA	1474
	Ser	Val	Leu	Ile	Gly	Ala	Phe	Ser	Val	Gly	Gln	Ala	Ser	Pro	
1475	AGC	ATT	GAA	GCA	TTT	GCA	AAT	GCA	AGA	GGA	GCA	GCT	TAT	GAA	1516
	Ser	Ile	Glu	Ala	Phe	Ala	Asn	Ala	Arg	Gly	Ala	Ala	Tyr	Glu	

TABLE 5 (cont'd.)

1517	ATC TTC AAG ATA ATT GAT AAT AAG CCA AGT ATT GAC AGC TAT Ile Phe Lys Ile Ile Asp Asn Lys Pro Ser Ile Asp Ser Tyr	1558
1559	TCG AAG AGT GGG CAC AAA CCA GAT AAT ATT AAG GGA AAT TTG Ser Lys Ser Gly His Lys Pro Asp Asn Ile Lys Gly Asn Leu	1600
1601	GAA TTC AGA AAT GTT CAC TTC AGT TAC CCA TCT CGA AAA GAA Glu Phe Arg Asn Val His Phe Ser Tyr Pro Ser Arg Lys Glu	1642
1643	GTT AAG ATC TTG AAG GGC CTG AAC CTG AAG GTG CAG AGT GGG Val Lys Ile Leu Lys Gly Leu Asn Leu Lys Val Gln Ser Gly	1684
1685	CAG ACG GTC GCC CTG GTT GGA AAC AGT GGC TGT GGG AAG AGC Gln Thr Val Ala Leu Val Gly Asn Ser Gly Cys Gly Lys Ser	1726
1727	ACA ACA GTC CAG CTG ATG CAG AGG CTC TAT GAC CCC ACA GAG Thr Thr Val Gln Leu MET Gln Arg Leu Tyr Asp Pro Thr Glu	1768
1769	GGG ATG GTC AGT GTT GAT GGA CAG GAT ATT AGG ACC ATA AAT Gly MET Val Ser Val Asp Gly Gln Asp Ile Arg Thr Ile Asn	1810
1811	GTA AGG TTT CTA CGG GAA ATC ATT GGT GTG AGT CAG GAA Val Arg Phe Leu Arg Glu Ile Ile Gly Val Val Ser Gln Glu	1852
1853	CCT GTA TTG TTT GCC ACC ACG ATA GCT GAA AAC ATT CGC TAT Pro Val Leu Phe Ala Thr Thr Ile Ala Glu Asn Ile Arg Tyr	1894
1895	GGC CGT GAA AAT GTC ACC ATG GAT GAG ATT GAG AAA GCT GTC Gly Arg Glu Asn Val Thr MET Asp Glu Ile Glu Lys Ala Val	1936

TABLE 5 (cont'd.)

1937	AAG GAA GCC AAT GCC TAT GAC TTT ATC ATG AAA CTG CCT CAT	1978
	Lys Glu Ala Asn Ala Tyr Asp Phe Ile MET Lys Leu Pro His	
1979	AAA TTT GAC ACC CTG GTT GGA GAG AGA GGG GCC CAG TTG AGT	2020
	Lys Phe Asp Thr Leu Val Gly Glu Arg Gly Ala Gln Leu Ser	
2021	GGT GGG CAG AAG CAG AGG ATC GCC ATT GCA CGT GCC CTG GTT	2062
	Gly Gly Gln Lys Gln Arg Ile Ala Ile Ala Arg Ala Leu Val	
2063	CGC AAC CCC AAG ATC CTC CTG CTG GAT GAG GCC ACG TCA GCC	2104
	Arg Asn Pro Lys Lys Ile Leu Leu Leu Asp Glu Ala Thr Ser Ala	
2105	TTG GAC ACA GAA AGC GAA GCA GTG GTT CAG GTG GCT CTG GAT	2146
	Leu Asp Thr Glu Ser Glu Ala Val Val Gln Val Ala Leu Asp	
2147	AAG GCC AGA AAA GGT CGG ACC ACC ATT CTG ATA GCT CAT CGT	2188
	Lys Ala Arg Lys Gly Arg Thr Thr Ile Val Ile Ala His Arg	
2189	TTG TCT ACA GTT CGT AAT GCT GAC GTC ATC GCT GGT TTC GAT	2230
	Leu Ser Thr Val Arg Asn Ala Asp Val Ile Ala Gly Phe Asp	
2231	GAT GGA GTC ATT GTG GAG AAA GGA AAT CAT GAT GAA CTC ATG	2272
	Asp Gly Val Ile Val Glu Lys Gly Asn His Asp Glu Leu MET	
2273	AAA GAG AAA GGC ATT TAC TTC AAA CTT GTC ACA ATG CAG ACA	2314
	Lys Glu Lys Gly Ile Tyr Phe Lys Leu Val Thr MET Gln Thr	
2315	GCA GGA AAT GAA GTT GAA TTA GAA AAT GCA GCT GAT GAA TCC	2356
	Ala Gly Asn Glu Val Glu Leu Glu Asn Ala Ala Asp Glu Ser	

TABLE 5 (cont'd.)

2357	AAA AGT GAA ATT GAT GCC TTG GAA ATG TCT TCA AAT GAT TCA Lys Ser Glu Ile Asp Ala Leu Glu MET Ser Ser Asn Asp Ser	2398
2399	AGA TCC AGT CTA ATA AGA AAA AGA TCA ACT CGT AGG AGT GTC Arg Ser Ser Leu Ile Arg Lys Arg Ser Thr Arg Arg Ser Val	2440
2441	CGT GGA TCA CAA GCC CAA GAC AGA AAG CTT AGT ACC AAA GAG Arg Gly Ser Gln Ala Gln Asp Arg Lys Leu Ser Thr Lys Glu	2482
2483	GCT CTG GAT GAA AGT ATA CCT CCA GTT TCC TTT TGG AGG ATT Ala Leu Asp Glu Ser Ile Pro Pro Val Ser Phe Thr Arg Ile	2524
2525	ATG AAG CTA AAT TTA ACT GAA TGG CCT TAT TTT GTT GGT MET Lys Leu Asn Leu Thr Glu Trp Pro Tyr Phe Val Val Gly	2566
2567	GTA TTT TGT GCC ATT ATA AAT GGA GGC CTG CAA CCA GCA TTT Val Phe Cys Ala Ile Ile Asn Gly Gly Leu Gln Pro Ala Phe	2608
2609	GCA ATA ATA TTT TCA AAG ATT ATA GGG GTT TTT ACA AGA ATT Ala Ile Ile Phe Ser Lys Ile Ile Gly Val Phe Thr Arg Ile	2650
2651	GAT GAT CCT GAA ACA AAA CCA CAG AAT AGT AAC TTG TTT TCA Asp Asp Pro Glu Thr Lys Arg Gln Asn Ser Asn Leu Phe Ser	2692
2693	CTA TTG TTT CTA GCC CTT GGA ATT ATT TCT TTT ATT ACA TTT Leu Leu Phe Leu Ala Leu Gly Ile Ile Ser Phe Ile Thr Phe	2734
2735	TTC CTT CAG GGT TTC ACA TTT GGC AAA GCT GGA GAG ATC CTC Phe Leu Gln Gly Phe Thr Phe Gly Lys Ala Gly Glu Ile Leu	2776

TABLE 5 (cont'd.)

2777	ACC AAG CGG CTC CGA TAC ATG GTT TTC CGA TCC ATG CTC AGA Thr Lys Arg Leu Arg Tyr MET Val Phe Arg Ser MET Leu Arg	2818
2819	CAG GAT GTG AGT TGG TTT GAT GAC CCT AAA AAC ACC ACT GGA Gln Asp Val Ser Trp Phe Asp Asp Pro Lys Asn Thr Thr Gly	2860
2861	GCA TTG ACT ACC AGG CTC GCC AAT GAT GCT GCT CAA GTT AAA Ala Leu Thr Thr Arg Leu Ala Ala Asn Asp Ala Ala Gln Val Lys	2902
2903	GGG GCT ATA GGT TCC AGG CTT GCT GTA ATT ACC CAG AAT ATA Gly Ala Ile Ile Gly Ser Arg Leu Ala Val Ile Thr Gln Asn Ile	2944
2945	GCA AAT CTT GGG ACA GGA ATA ATT ATA TCC TTC ATC TAT GGT Ala Asn Leu Leu Gly Thr Gly Ile Ile Ser Phe Ile Tyr Gly	2986
2987	TGG CAA CTA ACA CTG TTA CTC TTA GCA ATT GTA CCC ATC ATT Trp Gln Leu Thr Leu Leu Leu Ala Ile Val Pro Ile Ile	3028
3029	GCA ATA GCA GGA GTT GAA ATG AAA ATG TTG TCT GGA CAA Ala Ile Ala Gly Val Val Glu MET Lys MET Leu Ser Gly Gln	3070
3071	GCA CTG AAA GAT AAG AAA GAA CTA GAA GGT GCT GGG AAG ATC Ala Leu Lys Lys Asp Lys Lys Glu Leu Glu Gly Ala Gly Lys Ile	3112
3113	GCT ACT GAA GCA ATA GAA AAC TTC CGA ACC GTT GTT TCT TTG Ala Thr Glu Ala Ile Glu Asn Phe Arg Thr Val Val Ser Leu	3154
3155	ACT CAG GAG CAG AAG TTT GAA CAT ATG TAT GCT CAG AGT TTG Thr Gln Glu Glu Lys Phe Glu His MET Tyr Ala Gln Ser Leu	3196

TABLE 5 (cont'd.)

3197	CAG GTA CCA TAC AGA AAC TCT TTG AGG AAA GCA CAC ATC TTT Gln Val Pro Tyr Arg Asn Ser Leu Arg Lys Ala His Ile Phe	3238
3239	GGA ATT ACA TTT TCC TTC ACC CAG GCA ATG ATG TAT TTT TCC Gly Ile Thr Phe Ser Phe Thr Gln Ala MET MET Tyr Phe Ser	3280
3281	TAT GCT GGA TGT TTC CGG TTT GGA GCC TAC TTG GTG GCA CAT Tyr Ala Gly Cys Phe Arg Phe Gly Ala Tyr Leu Val Ala His	3322
3323	AAA CTC ATG AGC TTT GAG GAT GTT CTG TTA GTA TTT TCA GCT Lys Leu MET Ser Phe Glu Asp Val Leu Leu Val Phe Ser Ala	3364
3365	GTT GTC TTT GGT GCC ATG GCC GTG GGG CAA GTC AGT TCA TTT Val Val Phe Gly Ala MET Ala Val Gly Gln Val Ser Ser Phe	3406
3407	GCT CCT GAC TAT GCC AAA GCC AAA ATA TCA GCA GCC CAC ATC Ala Pro Asp Tyr Ala Lys Ala Lys Ile Ser Ala Ala His Ile	3448
3449	ATC ATG ATC ATT GAA AAA ACC CCT TTG ATT GAC AGC TAC AGC Ile MET Ile Ile Glu Lys Thr Pro Leu Ile Asp Ser Tyr Ser	3490
3491	ACG GAA GGC CTA ATG CCG AAC ACA TTG GAA GGA AAT GTC ACA Thr Glu Gly Leu MET Pro Asn Thr Leu Glu Gly Asn Val Thr	3532
3533	TTT GGT GAA GTT GTA TTC AAC TAT CCC ACC CGA CCG GAC ATC Phe Gly Glu Val Val Phe Asn Tyr Pro Thr Arg Pro Asp Ile	3574
3575	CCA GTG CTT CAG GGA CTG AGC CTG GAG GTG AAG GGC CAG Pro Val Leu Gln Gly Leu Ser Leu Glu Val Lys Lys Gly Gln	3616

TABLE 5 (cont'd.)

3617	ACG CTG GCT CTG GTG GGC AGC ACT GGC TGT GGG AAG AGC ACA Thr Leu Ala Leu Val Gly Ser Ser Gly Cys Gly Lys Ser Thr	3658
3659	GTG GTC CAG CTC CTG GAG CGG TTC TAC GAC CCC TTG GCA GGG Val Val Gln Leu Leu Glu Arg Phe Tyr Asp Pro Leu Ala Gly	3700
3701	AAA GTG CTG CTT GAT GGC AAA GAA ATA AAG CGA CTG AAT GTT Lys Val Leu Leu Asp Gly Lys Glu Ile Lys Arg Leu Asn Val	3742
3743	CAG TGG CTC CGA GCA CAC CTG GGC ATC GTG TCC CAG GAG CCC Gln Trp Leu Arg Ala His Leu Gly Ile Val Ser Gln Glu Pro	3784
3785	ATC CTG TTT GAC TGC AGC ATT GCT GAG AAC ATT GCC TAT GGA Ile Leu Phe Asp Cys Ser Ile Ala Glu Asn Ile Ala Tyr Gly	3826
3827	GAC AAC AGC CGG GTG TCA CAG GAA GAG ATC GTG AGG GCA Asp Asn Ser Arg Val Ser Gln Glu Ile Val Arg Ala	3868
3869	GCA AAG GAG GCC AAC ATA CAT GCC TTC ATC GAG TCA CTG CCT Ala Lys Glu Ala Asn Ile His Ala Phe Ile Glu Ser Leu Pro	3910
3911	AAT AAA TAT AGC ACT AAA GTA GGA GAC AAA GGA ACT CAG CTC Asn Lys Tyr Ser Thr Lys Val Gly Asp Lys Gly Thr Gln Leu	3952
3953	TCT GGT GGC CAG AAA CAA CGC ATT GCC ATA GCT GCC CTT Ser Gly Gly Gln Lys Gln Arg Ile Ala Ile Ala Arg Ala Leu	3994
3995	GTT AGA CAG CCT CAT ATT TTG CTT TTG GAT GAA GCC ACG TCA Val Arg Gln Pro His Ile Leu Leu Asp Glu Ala Thr Ser	4036

TABLE 5 (cont'd.)

4037	GCT CTG GAT ACA GAA AGT GAA AAG GTT GTC CAA GAA GCC CTG Ala Leu Asp Thr Glu Ser Glu Lys Val Val Gln Glu Ala Leu	4078
4079	GAC AAA GCC AGA GAA GGC CGC ACC TGC ATT GTG ATT GCT CAC Asp Lys Ala Arg Glu Glu Gly Arg Thr Cys Ile Val Ile Ala His	4120
4121	CGC CTG TCC ACC ATC CAG AAT GCA GAC TTA ATA GTG GTG TTT Arg Leu Ser Thr Ile Gln Asn Ala Asp Leu Ile Val Val Phe	4162
4163	CAG AAT GGC AGA GTC AAG GAG CAT GGC ACG CAT CAG CAG CTG Gln Asn Gly Arg Val Lys Glu His Gly Thr His Gln Gln Leu	4204
4205	CTG GCA CAG AAA GGC ATC TAT TTT TCA ATG GTC AGT GTC CAG Leu Ala Gln Lys Gly Ile Tyr Phe Ser MET Val Ser Val Gln	4246
4247	GCT GGA ACA AAG CGC CAG TGA 4267 Ala Gly Thr Lys Arg Gln TER	
4268	ACTCTCACTG TATCAGATGT TAAATACCTTT TTAATATTTG TTTAGATATG	
4318	ACATTTTATTC AAAGTTAAAA GCAACACTTT ACAGAATTAT GAAGAGGTAT	
4368	CTGTTTAACA TTTCCTCACT CAACTTCACA GTCTTCAGAG ACTTCGTAAT	
4418	TAAAGCAACA GAGTGAGAGA CATCATCAAG TGGAGAGAAA TCATAGTTTA	
4468	AACTCGATTA TAAATTTTAT AACAGAATPA AAGTAGATTT TAAAAGATAA	
4518	AATGTGTAAT TTTGTGTTATA TTTTCCCATTT TGGACTGTAA CTGACTGCCCT	
4568	TGCTAAAAGA TTATAGAAAGT AGCAAAAAGT ATTGAAAATGT TTGCATAAAG	
4618	TGCTTATAAT AAAACTAAAC TTTTCATGTGA AAAAAAAAAA AAAAAAAAAA	
4668	AA	

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Analysis of the amino acid sequence presented in Table 5 indicates that the mdr1 gene product is likely to be a transmembrane protein. This protein may consist of two approximately equal parts, with a
5 considerable sequence homology to each other, indicating that the mdr1 gene has likely evolved as a result of an internal duplication. Each half of the protein consists of a hydrophobic and a hydrophilic portion. Each of the hydrophobic portions includes six transmembrane domains,
10 as determined by the algorithm of Eisenberg et al. [J. Mol. Biol., 179, 125-142 (1984)]. Both hydrophilic portions contain two regions that share a high level of amino acid homology with the ATP-binding sites of several known enzymes. The best homology has been
15 observed with the ATP-binding sites of peripheral membrane components of bacterial periplasmic binding protein-dependent transport systems [Higgins et al., EMBO J., 4, 1033-1040, (1984)]. The presence of the transmembrane domains and potential glycosylation sites
20 within the protein sequence is consistent with the mdr1 protein being related to the P-glycoprotein, which is described above.

Analysis of the DNA and protein sequence information presented in Table 5 by the algorithm of
25 Eisenberg et al., supra, may be used to predict the protein regions that are located on the outside of the cell membrane. These protein regions may be produced either by chemical synthesis or by expression in the appropriate vector systems, and may be used to raise
30 antibodies against cells that express the mdr1 gene product, as described in Example 11.

Example 11

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The recombinant plasmids pMDR1 and pMDR2, as

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well as different individual fragments of recombinant plasmids pHDR4.4 and pHDR4.5, or the latter plasmids as a whole, or cDNA clones λ HDR5, λ HDR10, λ HDR62, λ HDR28 and λ HDR69, or other sequences according to the present invention, may be used as diagnostic tools for detection of human tumor cells resistant to chemotherapeutic drugs. These plasmids may be labeled directly with a radioactive isotope, according to the procedures of Rigby et al., Mol. Biol., 113, 237-251 (1977) or
10 Feinberg et al., Anal. Biochem., 132, 6-13 (1983), for example. Alternatively, the plasmids may be labelled with a non-radioactive chemical tag, for example, according to the procedure in Leary et al., Proc. Natl. Acad. Sci. (USA), 80, 4045-4049 (1983). The plasmids
15 may also be used to direct synthesis of labeled RNA probes [e.g., according to the procedure in Melton et al., Nucleic Acids Res., 12, 7035-7055 (1984)]. The labeled probes may then be used to detect the presence of homologous RNA sequences in tumor cells either by the
20 Northern hybridization procedure [according to Thomas, Proc. Natl. Acad. Sci. (USA), 77, 5201-5205 (1980)] or by dot blot or slot blot hybridization [according to Kafatos et al., Nucleic Acids Res., 7, 1541-1552 (1979) and Brown et al., Mol. Cell. Biol., 3, 1097-1107
25 (1983)], or by in situ hybridization techniques [e.g., those according to the procedures of Brahic et al., Proc. Natl. Acad. Sci. (USA), 75, 6125-6129 (1978)]. It is anticipated that in situ hybridization will provide a particularly sensitive method for detection of a small
30 number (1 in 1000 or fewer) of multidrug-resistant cells within a biopsy.

The mdr clones may be used to obtain polyclonal or monoclonal [Yelton et al., Ann. Rev. Biochem., 50, 657-680 (1981)] antibodies against mdr
35 gene products using either of two strategies.

The first strategy involves determination of

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the cDNA sequences of mdr genes, as described in Example 10. The cDNA sequence may be used to deduce the corresponding protein sequence. Peptides corresponding to different parts of mdr proteins, and preferably
5 comprising at least 15-20 amino acid residues, may be chemically synthesized by solid-phase methods [Marglin et al., Ann. Rev. Biochem., 39, 841-866 (1970)]. Such peptides may then be used to elicit specific polyclonal and monoclonal antibodies [Lerner, Nature, 299, 592-596
10 (1982); Niman et al., Proc. Natl. Acad. Sci. (USA), 80, 4949-4953 (1983)]. The availability of the full-length mdr1 cDNA sequence, as shown in Table 5, greatly facilitates the design of potentially immunogenic peptides, corresponding to different regions of the mdr1
15 protein, including the potential extracytoplasmic domains.

The second strategy involves expression of either complete or partial mdr gene products in bacteria, yeast or mammalian expression systems using
20 plasmid, phage or viral expression vectors [Vieira et al., Gene, 19, 259-268 (1982); Young et al., Proc. Natl. Acad. Sci. (USA), 80, 1194-1198 (1983); Bitter et al., Gene, 32, 263-274 (1984); Cepko et al., Cell, 37, 1053-62 (1984); and Gorman et al., Mol. Cell. Biol. 2, 1044-
25 1051 (1982)]. The expressed proteins may be purified and used in a vaccine or to raise specific antibodies. Antibodies against the mdr gene products may be used as the alternative diagnostic tools for detection of drug-resistant cells. Finally, such antibodies may
30 potentially be used as a basis for a new strategy of cancer immunotherapy. This strategy may involve, for example, conjugation of anti-mdr antibodies with radioactive isotopes or chemical toxins in order to specifically eliminate multidrug-resistant tumor cells. This
35 approach may be particularly efficient if used in combination with chemotherapy. Alternatively, the binding

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of anti-mdr antibodies to cells expressing mdr gene products, even in the absence of antibody-mediated cytotoxicity, may be sufficient to reverse the multidrug-resistant phenotype and may therefore render
5 tumor cells susceptible to the cytocidal action of the chemotherapeutic drugs.

In addition, complete or partial mdr gene products may be used as a vaccine to elicit an immune reaction in a patient against multidrug resistant tumor
10 cells.

Although the present invention has been described in terms of preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art upon consideration of the pre-
15 sent invention. Therefore, it is intended that all such equivalent variations and modifications should come within the scope of the invention as claimed.

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WHAT IS CLAIMED IS:

1. An isolated nucleic acid sequence for a
5 human mdr gene associated with multidrug resistance in
human cells.

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2. A nucleic acid selected from the group consisting of:

(a) a nucleic acid comprising a member of the group consisting of:

5 a continuous sequence of nucleotides as set forth in Table 4;

a continuous sequence of nucleotides as set forth in Table 5;

10 a continuous sequence of nucleotides as set forth in pHDR4.4 (ATCC 40227);

a continuous sequence of nucleotides as set forth in pHDR4.5 (ATCC 40228);

a continuous sequence of nucleotides as set forth in pHDR5A (ATCC 67040);

15 a continuous sequence of nucleotides as set forth in pHDR5B (ATCC 67041);

a continuous sequence of nucleotides as set forth in pHDR10 (ATCC 67042); and

20 a continuous sequence of nucleotides as set forth in pHDR104 (ATCC 67156);

(b) a nucleic acid comprising a nucleotide sequence which hybridizes with at least one of the continuous sequences of nucleotides as set forth in (a) or which is contained within the same mRNA molecule of
25 human origin or cDNA molecule of human origin as at least one of the continuous sequences of nucleotides as set forth in (a);

(c) a nucleic acid comprising a nucleotide sequence which hybridizes with any nucleotide sequences
30 described in (b); and

(d) nucleic acids comprising a sequence of nucleotides which, but for the degeneracy of the genetic code, would hybridize with at least one of the continuous sequences of nucleotides as set forth in (a), (b),
35 or (c).

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3. A nucleic acid probe comprising:

(a) a nucleic acid comprising a member of the group consisting of:

5 a continuous sequence of nucleotides as set forth in Table 4;

a continuous sequence of nucleotides as set forth in Table 5;

a continuous sequence of nucleotides as set forth in pHDR4.4 (ATCC 40227);

10 a continuous sequence of nucleotides as set forth in pHDR4.5 (ATCC 40228);

a continuous sequence of nucleotides as set forth in pHDR5A (ATCC 67040);

15 a continuous sequence of nucleotides as set forth in pHDR5B (ATCC 67041);

a continuous sequence of nucleotides as set forth in pHDR10 (ATCC 67042); and

a continuous sequence of nucleotides as set forth in pHDR104 (ATTC 67156);

20 (b) a nucleic acid comprising a nucleotide sequence which hybridizes with at least one of the continuous sequences of nucleotides as set forth in (a) or which is contained within the same mRNA molecule of human origin or cDNA molecule of human origin as at least one of the continuous sequences of nucleotides as set forth in (a);

(c) a nucleic acid comprising a nucleotide sequence which hybridizes with any nucleotide sequences described in (b); and

30 (d) nucleic acids comprising a sequence of nucleotides which, but for the degeneracy of the genetic code, would hybridize with at least one of the continuous sequences of nucleotides as set forth in (a), (b), or (c); and

35 a label associated with said polynucleotide.

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4. A polypeptide comprising a continuous sequence of amino acids encoded by a nucleic acid selected from the group consisting of:

(a) a nucleic acid comprising a member of the group consisting of:

a continuous sequence of nucleotides as set forth in Table 4;

a continuous sequence of nucleotides as set forth in Table 5;

10 a continuous sequence of nucleotides as set forth in pHDR4.4 (ATCC 40227);

a continuous sequence of nucleotides as set forth in pHDR4.5 (ATCC 40228);

15 a continuous sequence of nucleotides as set forth in pHDR5A (ATCC 67040);

a continuous sequence of nucleotides as set forth in pHDR5B (ATCC 67041);

a continuous sequence of nucleotides as set forth in pHDR10 (ATCC 67042); and

20 a continuous sequence of nucleotides as set forth in pHDR104 (ATCC 67156);

(b) a nucleic acid comprising a nucleotide sequence which hybridizes with at least one of the continuous sequences of nucleotides as set forth in (a) or
25 which is contained within the same mRNA molecule of human origin or cDNA molecule of human origin as at least one of the continuous sequences of nucleotides as set forth in (a);

(c) nucleic acids comprising a nucleotide
30 sequence which hybridizes with any nucleotide sequences described in (b); and

(d) nucleic acids comprising a sequence of nucleotides which, but for the degeneracy of the genetic code, would hybridize with at least one of the continuous sequences of nucleotides as set forth in (a), (b),
35 or (c).

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5. A composition effective as a vaccine or as an antigen for induction of specific antibodies, comprising a polypeptide as recited in claim 4 and a compatible diluent, adjuvant, or carrier.

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6. An antibody to a polypeptide comprising a continuous sequence of amino acids encoded by a nucleic acid selected from the group consisting of:

(a) a nucleic acid comprising a member of the
5 group consisting of:

a continuous sequence of nucleotides as set forth in Table 4;

a continuous sequence of nucleotides as set forth in Table 5;

10 a continuous sequence of nucleotides as set forth in pHDR4.4 (ATCC 40227);

a continuous sequence of nucleotides as set forth in pHDR4.5 (ATCC 40228);

15 a continuous sequence of nucleotides as set forth in pHDR5A (ATCC 67040);

a continuous sequence of nucleotides as set forth in pHDR5B (ATCC 67041);

a continuous sequence of nucleotides as set forth in pHDR10 (ATCC 67042); and

20 a continuous sequence of nucleotides as set forth in pHDR104 (ATCC 67156);

(b) a nucleic acid comprising a nucleotide sequence which hybridizes with at least one of the continuous sequences of nucleotides as set forth in (a) or
25 which is contained within the same mRNA molecule of human origin or cDNA molecule of human origin as at least one of the continuous sequences of nucleotides as set forth in (a);

(c) a nucleic acid comprising a nucleotide
30 sequence which hybridizes with any nucleotide sequences described in (b); and

(d) nucleic acids comprising a sequence of nucleotides which, but for the degeneracy of the genetic code, would hybridize with at least one of the continuous
35 sequences of nucleotides as set forth in (a), (b), or (c).

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7. A diagnostic reagent comprising:

an antibody to a polypeptide comprising a continuous sequence of amino acids encoded by a nucleic acid selected from the group consisting of:

- 5 (a) a nucleic acid comprising a member of the group consisting of:

a continuous sequence of nucleotides as set forth in Table 4;

- 10 a continuous sequence of nucleotides as set forth in Table 5;

a continuous sequence of nucleotides as set forth in pHDR4.4 (ATCC 40227);

a continuous sequence of nucleotides as set forth in pHDR4.5 (ATCC 40228);

- 15 a continuous sequence of nucleotides as set forth in pHDR5A (ATCC 67040);

a continuous sequence of nucleotides as set forth in pHDR5B (ATCC 67041);

- 20 a continuous sequence of nucleotides as set forth in pHDR10 (ATCC 67042); and

a continuous sequence of nucleotides as set forth in pHDR104 (ATCC 67516);

- 25 (b) a nucleic acid comprising a nucleotide sequence which hybridizes with at least one of the continuous sequences of nucleotides as set forth in (a) or which is contained within the same mRNA molecule of human origin or cDNA molecule of human origin as at least one of the continuous sequences of nucleotides as set forth in (a);

- 30 (c) a nucleic acid comprising a nucleotide sequence which hybridizes with any nucleotide sequences described in (b); and

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(d) nucleic acids comprising a sequence of nucleotides which, but for the degeneracy of the genetic code, would hybridize with at least one of the continuous sequences of nucleotides as set forth in (a), (b),
5 or (c); and

a label associated with said monoclonal antibody.

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8. An immunotherapeutic preparation comprising:

an antibody to a polypeptide comprising a continuous sequence of amino acids encoded by a nucleic acid selected from the group consisting of:

(a) a nucleic acid comprising a member of the group consisting of:

a continuous sequence of nucleotides as set forth in Table 4;

10 a continuous sequence of nucleotides as set forth in Table 5;

a continuous sequence of nucleotides as set forth in pHDR4.4 (ATCC 40227);

15 a continuous sequence of nucleotides as set forth in pHDR4.5 (ATCC 40228);

a continuous sequence of nucleotides as set forth in pHDR5A (ATCC 67040);

a continuous sequence of nucleotides as set forth in pHDR5B (ATCC 67041);

20 a continuous sequence of nucleotides as set forth in pHDR10 (ATCC 67042); and

a continuous sequence of nucleotides as set forth in pHDR104 (ATCC 67156);

(b) a nucleic acid comprising a nucleotide sequence which hybridizes with at least one of the continuous sequences of nucleotides as set forth in (a) or which is contained within the same mRNA molecule of human origin or cDNA molecule of human origin as at least one of the continuous sequences of nucleotides as set forth in (a);

30 (c) a nucleic acid comprising a nucleotide sequence which hybridizes with any nucleotide sequences described in (b); and

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(d) nucleic acids comprising a sequence of nucleotides which, but for the degeneracy of the genetic code, would hybridize with at least one of the continuous sequences of nucleotides as set forth in (a), (b),
5 or (c).

9. The immunotherapeutic preparation as recited in Claim 8 further comprising a cytocidal agent conjugated with said antibody.

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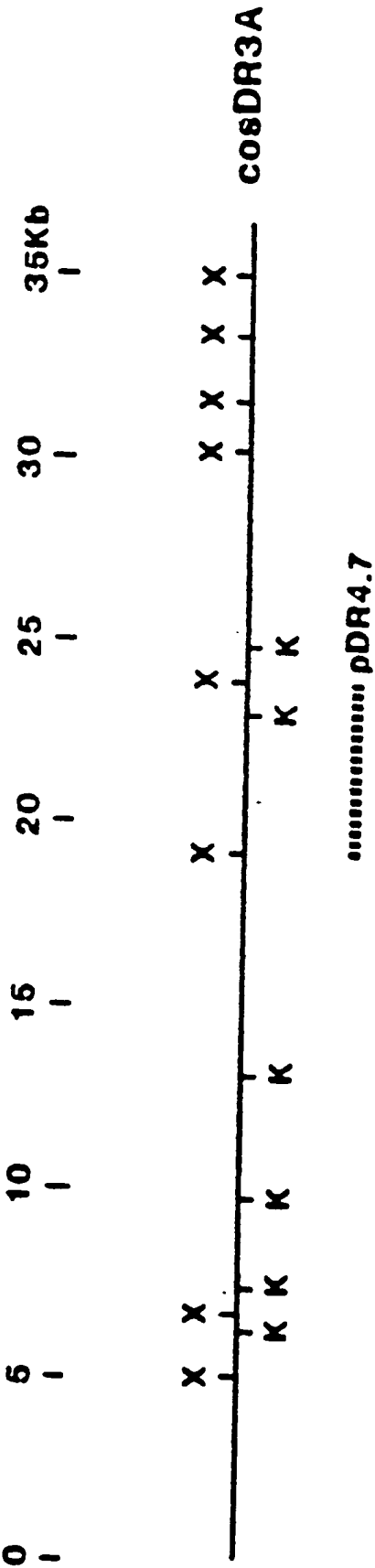


FIG. 1

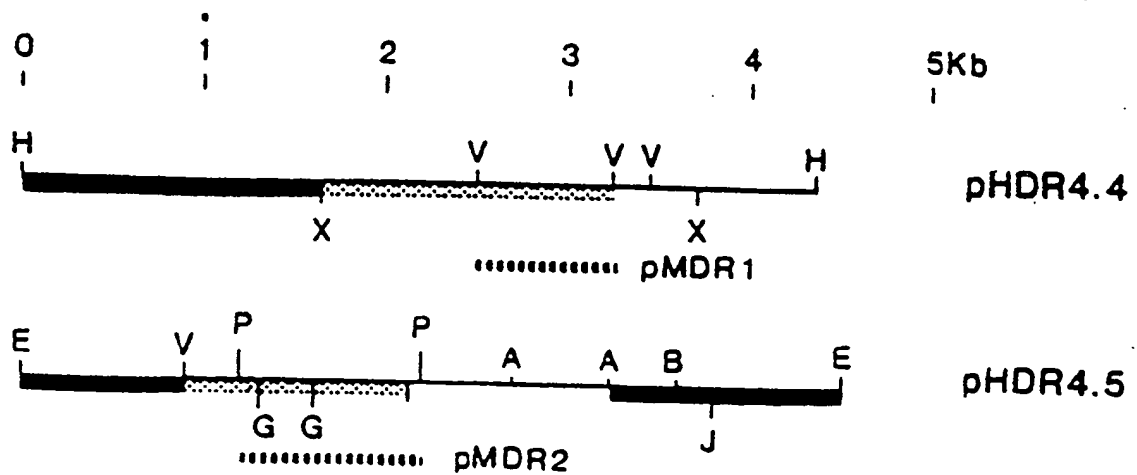
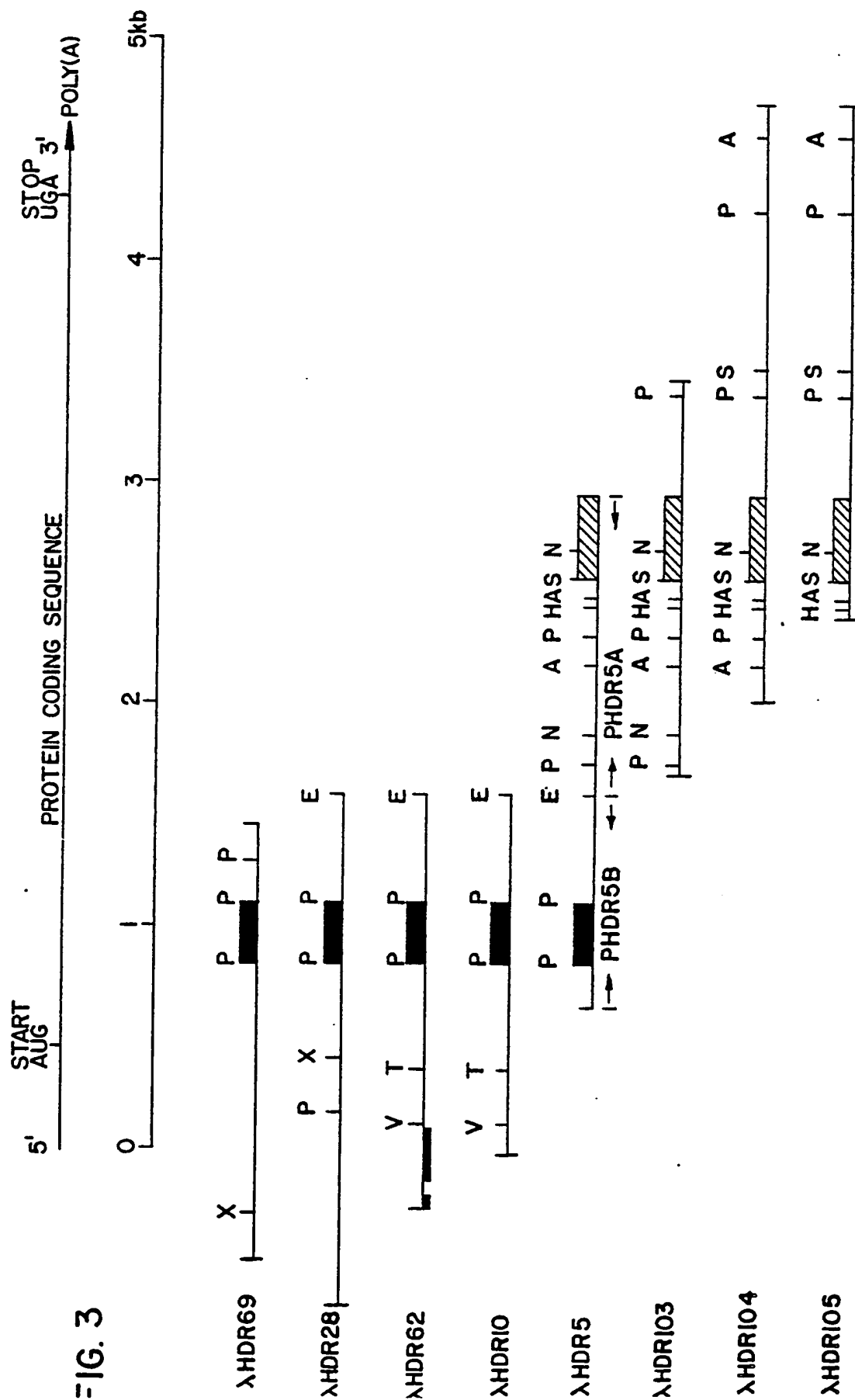


FIG. 2



INTERNATIONAL SEARCH REPORT

International Application No **PCT/US87/00758**

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl. (4): **C12Q 1/68**

U.S. Cl.: **435/6**

II. FIELDS SEARCHED

Minimum Documentation Searched ⁴

Classification System |

Classification Symbols

U.S. **435/6; 536/27**
 935/78, 9
 436/501

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁵

Computer Search: **Lexpat; APS; Chemical Abstracts 1967-1987;**
Biosis 1977-1987

III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴

Category [*]	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
Y	Proceedings National Academy of Sciences U.S.A., Vol. 82, No. 22, issued November, 1985 (Washington D.C., USA) A.T. FOJO ET AL. "Amplification of DNA sequences in human multidrug-resistant KB carcinoma cells", see pages 7661 and 7665.	1-3
Y	Proceedings National Academy of Sciences, U.S.A., Vol. 83 No. 2, issued January 1986 (Washington D.C., USA) P. CROSS ET AL, "Isolation and characterization of DNA sequences amplified in multidrug-resistant hamster cells", see page 337.	1-3
P,X	Chemical Abstracts, Vol. 106, No. 9, issued 02 March 1987 (Columbus, OH, USA) K. UEDA ET AL, "The mdrl gene, responsible for multidrug-resistance, codes for p-glycoprotein" see page 154, column 1, the abstract No. 62136q, Biochem. Biophys. Res. Commun. 1986, 141 (3) 956-62 (Eng.)	1-3

^{*} Special categories of cited documents: ¹⁵

"A" document defining the general state of the art which is not
considered to be of particular relevance

"E" earlier document but published on or after the international
filing date

"L" document which may throw doubts on priority claim(s) or
which is cited to establish the publication date of another
citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or
other means

"P" document published prior to the international filing date but
later than the priority date claimed

"T" later document published after the international filing date
or priority date and not in conflict with the application but
cited to understand the principle or theory underlying the
invention

"X" document of particular relevance; the claimed invention
cannot be considered novel or cannot be considered to
involve an inventive step

"Y" document of particular relevance; the claimed invention
cannot be considered to involve an inventive step when the
document is combined with one or more other such docu-
ments, such combination being obvious to a person skilled
in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search ¹

03 JUNE 1987

International Searching Authority ¹

Date of Mailing of this International Search Report ²

11 JUN 1987

Signature of Authorized Officer ²⁰

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

P, X: Chemical Abstracts Vol. 105, No. 7, issued
18 August 1986 (Columbus, OH, USA)
I.B. RONINSON ET AL, "Isolation of human mdr
DNA sequences amplified in multidrug-resistant
KB carcinoma cells" see page 165, column 2, the
abstract No. 55593n, Proc. Natl. Acad. Sci.
U.S.A. 1986 83(12) 4538-42 (Eng.)

1-3

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹⁰

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers because they relate to subject matter ¹² not required to be searched by this Authority, namely:

2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹³, specifically:

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ¹¹

This International Searching Authority found multiple inventions in this international application as follows:

See Attachment.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers: 1-3.

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No ¹⁸
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P,X	Chemical Abstracts, Vol. 105, No. 3, issued 21 July 1986 (Columbus, OH, USA) J.R. RIORDAN ET AL, "Multidrug resistance in mammalian cell lines and isolation of determinant glycoprotein DNA" see page 226, column 1, the abstract No. 19855r, Eur. Pat. Appl. EP 174,810 19 MARCH 1986, GB Appl. 84/22,819 10 September 1984; 34 pp.	1-3
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Y	Chemical Abstracts, Vol. 101, No. 7, issued 13 August 1984 (Columbus, OH, USA) V. LING ET AL, "DNA-mediated transfer of multidrug-resistance and expression of P-glycoprotein" see page 145, column 1, the abstract No. 49,442p, Prog. Cancer Res. Ther. 1984 30, 53-7 (Eng.)	1-3
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VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING
CONTINUED

- I. Claims 1-3, drawn to nucleic acid sequences and probes to detect a gene; class 435 subclass 6 and class 536 subclass 27.
- II. Claims 5 and 8, drawn to a vaccine and immunotherapeutic preparation; class 424 subclasses 85 and 88.
- III. Claims 4, 6 and 7, drawn to a polypeptide, an antibody against the polypeptide and a diagnostic reagent; class 436 subclass 518.

The above inventions lack unity under PCT Rule 13 since each is used for an entirely different method (i.e., hybridization, vaccination and an immunoassay).